

HYPOGLYCAEMIC AND ANTI-OXIDANT ACTIVITY OF SALACIA OBLONGA WALL. EXTRACT IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract : The petroleum ether extract of the root bark of *S. oblonga* Wall. (Celastraceae) (SOB) was studied in streptozotocin (STZ) diabetic rats and anti-lipid peroxidative activity of the same was studied in the cardiac tissue. SOB prevented significantly the streptozotocin-induced hyperglycaemia and hypoinsulinaemia. It also produced a significant decrease in peroxidation products viz. thiobarbituric acid reactive substances ($P < 0.001$), conjugated dienes ($P < 0.05$), hydroperoxides ($P < 0.001$). The activity of antioxidant enzymes such as superoxide dismutase ($P < 0.001$), catalase ($P < 0.001$), GSHPxase and GSSGRase was found to be increased in the heart tissue of diabetic animals treated with SOB. These results suggest that *S. oblonga* root bark extract possesses anti-diabetic and anti-oxidative activity in streptozotocin-diabetic rats.

Key words : *Salacia oblonga* hypoglycaemia lipid peroxidation
antioxidant enzymes streptozotocin diabetes

INTRODUCTION

S. oblonga Wall. (Celastraceae) is commonly known as 'ponkoranti' in Malayalam due to its golden coloured root bark. In Indian traditional medicine, the root bark of *S. oblonga* is used in gonorrhoea, rheumatism and skin diseases (1, 2, 3). The aqueous extract of the root bark has shown hypoglycaemic activity

(4, 5). Root bark boiled in oil or as decoction or as powder is used for the treatment of rheumatism, gonorrhoea, itches, asthma, thirst and ear diseases (6). Oral administration of root bark powder showed anti-inflammatory activity (7). In the light of the above reports the anti-diabetic activity of *S. oblonga* root bark in hyperglycaemic rats was studied.

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As there is growing literature on the toxic effects of oxygen free radicals and cardiovascular complications (8) especially in diabetic condition (9) and to determine the mechanism of action of this drug, the level of lipid peroxides viz. thiobarbituric acid reactive substances, conjugated dienes, hydroperoxides, antioxidant glutathione content, the related antioxidant enzymes superoxide dismutase (SOD), catalase, GSH Pxase, GSSG Rase etc. in the cardiac tissue were also studied.

METHODS

Animals

Female albino rats (Sprague-Dawley strain) from inbred stock having average body weight 200 g were used for the experiments.

Plant material and extraction

S. oblonga root was collected from Western Ghats (8° 13' 38" N and 77° 30' 16" E, Altitude-505 m), Thovalai, Kanyakumari District, Tamil Nadu, South India. The bark was removed from the root (yield 150 g/kg) and powdered in an electric grinder. The powder of bark was subjected to Soxhlet extraction for 16 h with the following solvents sequentially: petroleum ether (60–80), chloroform, acetone, methanol and water (10). The yield of the extract was 2.81, 0.02, 5.11, 2.66, 16.00% (w/w in terms of dried starting material) respectively.

Experimental diabetes

Diabetes was induced by 65 mg/kg of streptozotocin administered i.p. in saline

with the pH having been adjusted to 4.5 with 0.05M citric acid (11). After one week glucose was measured in blood samples collected from orbital plexus. Rats with glucose levels above 200 mg/dl were included in the experiment.

Experimental procedure

The animal were divided into 5 groups of 6 animals each. Group I control rats injected with 0.5 ml of physiological saline, group II rats were streptozotocin treated rats, group III diabetic rats given petroleum ether extract orally (hereafter referred to as SOB) 250 mg/kg of body weight daily, group IV diabetic rats received glibenclamide (Hoechst, India) orally (600 µg/kg/day) and group V diabetic rats received bovine insulin (Knoll Pharma, India) subcutaneously (6 units/kg). The duration of the experiment was one month. At the end of the period the animals were deprived of food overnight and sacrificed by decapitation.

Estimation of glucose and insulin

Blood glucose was determined by the glucose oxidase method (12). The insulin assay was done with a radioimmunoassay kit (Coat-A-Count®, DPC, Los Angeles) by the method of Morgan and Lazarow (13).

Estimation of peroxidation products and antioxidants

The levels of peroxidation products viz. thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD) and hydroperoxides (HP), glutathione (GSH) and

activity of antioxidant enzymes: superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione reductase (GSSGRase, EC 1.6.4.2) and glutathione peroxidase (GSHPxase, EC 1.11.1.9) were estimated in the cardiac tissue as reported earlier (14). The protein content of the enzyme extract was determined by Lowry's method (15).

Data analyses

Statistical analysis was done using the student's 't' test for glucose estimation, other parameters were analysed by one way analysis of variance (ANOVA) and difference between treatment means were determined by Bonferroni multiple comparison procedure.

Drugs and chemicals

All the biochemicals used in this experiment were purchased from Sigma Chemical Company Inc., St. Louis, MO, USA unless otherwise specified. The chemicals were of analytical grade.

RESULTS

Blood glucose

The hyperglycaemic animals showed significant decrease in the blood glucose level on long term treatment for one month with SOB at a dose of 250 mg/kg (Group III). The glibenclamide and insulin treatment also produced significant decrease in glucose levels in streptozotocin-diabetic rats. The activity of SOB was about 65.5% of glibenclamide activity and 49.9% of the insulin. The serum levels of insulin were increased significantly in SOB treated group (Table I).

Lipid peroxide concentration

The concentration of TBARS, CD and HP were significantly decreased in the SOB treated group as compared to the diabetic control (Table I).

Glutathione content

The GSH content was increased in the

TABLE I : Concentration of lipid peroxides, blood glucose, glutathione and insulin.

Group	TBARS*	Conjugated dienes*	Hydroperoxides*	GSH*	Serum Glucose**	Serum Insulin***
I	0.402±0.01	15.12±0.54	13.68±0.37	405.48±13.9	78.2±5.9	75.36±5.8
II	0.521±0.01 ^a	20.01±0.67 ^a	18.15±0.51 ^a	357.86±9.5 ^a	387.0±29.3 ^a	24.55±2.0 ^a
III	0.476±0.01 ^b	18.96±0.65 ^c	16.26±0.80 ^b	379.31±12.9 ^c	197.2±14.0 ^b	58.08±4.9 ^b
IV	0.445±0.01 ^b	18.89±0.67 ^c	15.51±0.92 ^b	383.41±13.0 ^c	153.6±11.1 ^b	59.93±4.9
V	0.436±0.01 ^b	17.10±0.59 ^b	14.61±0.42 ^b	391.21±13.2 ^c	107.3±7.2 ^b	-

Each value is the mean ± S.D of 6 rats.

^aP<0.001 compared with group I, ^bP<0.001 compared with group II, ^cP<0.05 compared with group II, *(mM/100 g wet tissue), **mg/dl, ***(μ U/ml).

TABLE II : Activity of the antioxidant enzymes.

Group	SOD*	Catalase**	GSH Pxase***	GSSG Rase****
I	12.99±0.35	8.16±0.54	0.79±0.11	4.46±0.57
II	7.09±0.06 ^a	5.18±0.15 ^a	0.67±0.08 ^a	2.94±0.37 ^a
III	8.04±0.21 ^b	5.95±0.38 ^b	0.70±0.09	3.04±0.39
IV	8.27±0.22 ^b	6.85±0.44 ^b	0.71±0.10	3.85±0.49
V	9.37±0.23 ^b	7.05±0.45 ^b	0.86±0.12 ^b	3.34±0.43 ^b

Each value is the mean ± S.D of 6 rats.

*P<0.001 compared with group I, ^aP<0.001 compared with group II

*Units (Enzyme concentration required to inhibit chromogen production (O.D. of 560 nm) by 50% in one minute)/mg protein.

Values x 10⁻³ units ((Velocity constant/second/mg protein). *Units (µmoles of NADPH oxidised/minute/mg protein.

****Values x 10⁻² units (µmoles of NADPH oxidised/minute/mg protein.

SOB, glibenclamide and insulin groups as compared to diabetic control (Table I).

Activity of anti-oxidant enzymes

The activity of SOD and catalase was significantly increased in treated groups (Group III, IV and V). But the activity of enzymes GSHPxase and GSSGRase showed significant increase in insulin and glibenclamide treated groups, but non-significant changes in SOB treated group (Table II).

DISCUSSION

The experiments reveal that SOB (250 mg/kg) significantly (P<0.001) decreased the blood glucose level in hyperglycaemic animals. The glucose lowering activity observed in the diabetic animals may be due to the stimulation of the β-cells of the pancreatic islets because insulin levels were found to be increased by SOB in streptozotocin-diabetic rats as compared to control.

Karpen et al. (16) observed an elevated level of lipid peroxides in the plasma of

streptozotocin diabetic rats and lipid peroxidation is one of the characteristic features of chronic diabetes (17). The increased levels of thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD) and hydroperoxides are indices of lipid peroxidation. The levels of TBARS, CD and HP were decreased significantly in the cardiac tissue of rats given SOB, glibenclamide and insulin as compared to the diabetic control animals where the levels were quite high (Table I). This indicates that SOB may inhibit oxidative damage of the cardiac tissue.

Under *in vivo* condition, glutathione (GSH) acts as an antioxidant and its decrease is reported in diabetes mellitus (18). The increased GSH content (P<0.05) in the heart of the rats treated with SOB may be one of the factors responsible for the inhibition of lipid peroxidation. Superoxide dismutase and catalase are the two major scavenging enzymes that remove the toxic free radicals *in vivo*. Vucic et al. (19) reported that the activity of SOD is low in diabetes mellitus. The SOB treated rats showed decreased lipid peroxidation

associated with increased activity of SOD and catalase (Table II). The antioxidant enzymes GSH Pxase and GSSG Rase showed only non-significant differences in SOB treated group. However, the results shows the positive anti-peroxidative activity of *S. oblonga*.

In conclusion, the above observations show that the petroleum ether soluble fraction of *S. oblonga* root bark (SOB)

possess antidiabetic as well as antioxidant principle and it may prevent the cardiovascular complications that may arise due to lipid peroxidation in cardiac tissues especially in diabetic conditions.

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